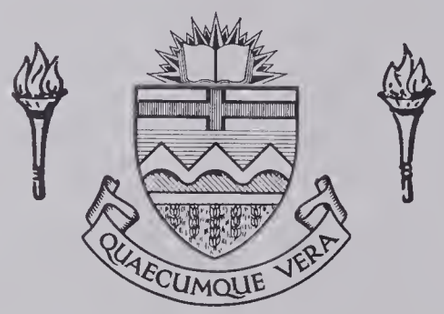

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KARYOTYPIC AND CYTOTAXONOMIC STUDY OF DOMESTIC CATTLE AND AMERICAN BISON

by

© RAMESH N. BHAMBHANI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

JULY, 1968

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Karyotypic and Cyto-taxonomic Study of Domestic Cattle and American Bison," submitted by Ramesh N. Bhambhani, in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Karyotypic comparisons are often utilised as a criterion in the assessment of cytotaxonomic relationships of closely related forms. In some instances, such comparative cytological studies have helped confirm the conclusions of the taxonomist. This investigation is concerned with an assessment of the cytotaxonomic relationship that exists between two bovine forms viz. Bos taurus (L) and Bos (Bison) bison (L) members of the family Bovidae.

On the basis of morphological criteria alone, the domestic cattle and the American bison have been placed in distinct sub-genera viz. Bos and Bison. This investigation, wherein the karyotypes are compared, reveals that the two forms differ only slightly with respect to their somatic complements. Specifically, the heterogametic sexes of the two forms differ with respect to their Y-chromosomes. Purely on the basis of measurements (relative percentage lengths of the chromosome pairs) and gross morphological similarities, it is suggested that the chromosomes are homologous. The identity in number and similarity in morphology of the chromosomes also suggests that the two bovine forms may be placed within the single genus Bos. For further confirmation of the above conclusions, it is essential that the meiotic chromosome behaviour of the interspecific hybrid between the two forms, be studied.

The investigation utilised a peripheral-blood culture method to obtain the somatic karyotypes of the above bovine forms.

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ACKNOWLEDGEMENTS

I wish to sincerely thank Dr. J. Kuspira for advice, encouragement and discussion throughout this study. I express my gratitude to Dr. G.W.R. Walker for his sagacious criticisms and helpful suggestions in the compiling of this thesis.

Sincere thanks are extended to Dr. R.E. Giblak for his invaluable technical advice and correction of this manuscript. Special thanks are extended to Mr. D.C. Wighton and Mr. J. Dietrich for their valuable assistance in the preparation of the karyotypes.

I am indebted to Dr. R.E. Gilchrist for his assistance with the collection of the bison blood samples. The co-operation of Gainers Co. Ltd., and the staff of Elk Island National Park is gratefully acknowledged.

Humble thanks are extended to Mrs. P. Brunel who assisted in the typing of this manuscript. The ever-available assistance of the members of the Department of Genetics is also acknowledged.

The study was supported by a grant from the National Research Council of Canada to Dr. J. Kuspira.

INTRODUCTION

With the recent development of refined mammalian cytological techniques, cytology has received more attention by animal taxonomists than in the past; and it has now become an important facet of their taxonomic investigations.

The karyotype has been defined as a systematised array of the chromosomes of a single cell, prepared either by drawing or by photography, with the extension in meaning that the chromosomes of the single cell typify the chromosomes of an individual or even a species (International Study Group, 1960). All species are characterised by specific and distinct karyotypes, which, with few exceptions (for example, three species of the family Camelidae (Taylor et al, 1968); the two members of the family Bovidae that are being considered in this study and a few others), are unlike those of other species. The karyotype therefore constitutes the visible manifestation of a species' genetic system. The comparison of karyotypes of closely related species may reveal morphological differences and similarities between their genomes. These karyotypic properties may in turn suggest possible genetic relationships amongst the species (Chu and Bender, 1962). Karyotypic comparisons may reveal mechanisms of chromosomal evolution, as has been illustrated in the genus Drosophila (Patterson and Stone, 1952). The results of comparative studies of chromosomal morphology suggest an intermediate stage in evolution, that cannot be detected using classical taxonomic criteria (Nadler, 1964). Consequently, karyotypic

comparisons are now frequently utilised as criteria for evaluating evolutionary relationships.

Karyological research in animals, prior to the mid-1950s, was restricted almost exclusively to non-mammalian organisms (amphibians, fish, insects), since adequate pretreatment methods, necessary for the accurate description of the number and morphology of chromosomes of a species, were not available. New technical advances were introduced during the mid-1950s. The utilisation of tissue culture techniques in cytology, combined with the use of hypotonic solution pretreatment to spread the chromosomes and colchicine to contract them, has provided investigators with refined techniques for karyotypic analysis. This prompted keen interest in the field of karyological research and several investigators have turned their attention to such studies in a variety of mammalian organisms.

Despite the technical improvements, investigations of this nature, with agronomically important animals, have been limited. Domestic cattle and the potentially important American bison (two members of the family Bovidae), were experimentally hybridised as early as 1914 (Boyd, 1914). This was done in an attempt to produce a breed of beef-cattle that would combine the winter hardiness of the American bison and the beef-producing ability of the domestic animal. Extensive hybridisation experiments for economic purposes have since been conducted by the Canadian Federal Government (Peters, 1964). Such efforts emphasized the necessity of studies to clarify the causes of hybrid sterility and the

failure of certain crosses. Therefore, a comparison of the genetic systems of the two forms [Bos taurus (domestic cattle) and Bos bison (American bison)] was decided upon. This was to be achieved by studying chromosome morphology and chromosome behaviour during mitosis in the two species and during both mitosis and meiosis in the interspecific hybrid. Unfortunately, the experimental hybrids were recently slaughtered at Manyberries, Alberta, when the Canadian Department of Agriculture disbanded the project. Attempts to obtain the hybrid animal from elsewhere were unsuccessful and therefore precluded any such study. Consequently, the somatic karyotype (as defined earlier) was determined for each species; and on the basis of a karyotypic comparison, the cytotaxonomic relationship(s) that may or may not exist between the two species were assessed.

LITERATURE REVIEW

Various criteria, other than those used in classical systematics, can be utilised to demonstrate phylogenetic relatedness. Currently, taxonomic relatedness is frequently demonstrated in related forms, by a direct comparison of their karyotypes.

Unreliable procedures in mammalian cytology prior to the mid-1950s precluded such comparisons. Recent technical and theoretical advances in tissue culture have resulted in reliable chromosome preparations, which can be used for karyotypic analyses and comparisons. In fact, the utilisation of the various tissue culture methods has permitted an extensive revision of the chromosome karyotypes of various species. For example, the diploid chromosome number in man had been reported as 48, prior to 1956 (Hsu, 1952 and Painter, 1921). In that year it was correctly identified as 46, by Tjio and Levan in primary tissue cultures of fibroblasts explanted from embryos; and by Ford and Hamerton in the gonadal tissues of adult males.

Haldane (1932) predicted that lymphocytes would be cultured for the purpose of chromosome analysis. This prediction was rendered prophetic with the fortuitous discovery, when lymphocytes were cultured in the presence of phytohemagglutinin (PHA) -- a red kidney bean (*Phaseolus vulgaris*) extract (Hungerford *et al*, 1959). This *in vitro* method of peripheral blood culture, utilising PHA-stimulated lymphocytes, has received considerable attention since its casual discovery. Various modifications of the original technique are constantly being suggested.

In the present study certain alterations (see Materials and Methods) were deemed necessary before the technique could be successfully utilised. Despite its universal use in cytology, certain aspects of the technique still remain enigmas. These will now be given due consideration.

Under suitable *in vitro* conditions, peripheral lymphocytes from humans as well as several other animal species, undergo a morphological transformation ("blastogenesis") and subsequent mitoses in the presence of phytohemagglutinin (PHA) (Hungerford *et al*, 1959; Basrur and Gilman, 1964; Moorhead *et al*, 1960; Evans, 1965; Nichols and Levan, 1962 and others). However, the exact mechanism of action of PHA in peripheral blood cultures still remains undeciphered. The precise chemical nature of the "active factor" in the bean extract causing transformation in lymphocyte morphology, has yet to be defined. Some authors state that "blastogenesis" is the direct consequence of PHA attachment to some specific structure of the small lymphocyte *viz*. the cell membrane or nucleus (Nowell, 1960a; Hirschhorn *et al*, 1963).

The mitogenic potentials of the extract were accidentally realised by Hungerford *et al* (1959), who used a modified version of the Li and Osgood (1949) extract to culture cells for the examination of human chromosomes. It is suggested that the mitogenic effects are probably due to the ability of PHA to cause a rejuvenation of the lymphocytes. This in turn causes a general increase in metabolism and DNA synthesis. Ultimately, this results in the division of some cells (Elves and Wilkinson, 1962). Another suggestion is that the reaction may be similar, but

not identical to the development of an immunological response by the lymphocyte to specific antigens (Pearmain et al, 1963).

Inclusion of "heat inactivated" serum as a necessary constituent in the culture medium also remains an enigma. There is some evidence that actual utilisation of protein occurs in tissue cultures (Tritsch et al, 1966). Tritsch (1967), showed that different cell lines in culture have different quantitative and qualitative protein requirements to maintain growth. He suggested that the serum contains a broad spectrum of growth factors (probably proteins) that can satisfy the requirements of a number of cell lines.

The reasoning behind the use of the drug colchicine is well established. The drug is known to interfere with the formation of the mitotic spindle, ultimately resulting in mitotic arrest at metaphase (Gavaudan et al, 1937; Tjio and Levan, 1956; Ford and Hamerton, 1956b). Colchicine arrest can take place without inhibiting any one of the sequential steps in cell division, such as DNA replication, RNA or protein synthesis (Borisy and Taylor, 1967). In studies with sea urchin eggs they found that the colchicine molecule was bound to a protein molecule, which turned out to be a micro-tubule sub-unit. Therefore they suggested that the binding of colchicine, prevents the assembly of protein sub-units into micro-tubules and this leads to a dis-orientation and possible disaggregation of the mitotic apparatus (Borisy and Taylor, 1967).

The use of hypotonic solutions is necessary for the swelling of the cells and the dispersion of metaphase chromosomes (Hsu, 1952).

As a refinement of Hungerford's technique, Moorhead et al (1960) combined peripheral blood culture with the air-drying method of Rothfels and Siminovitch (1958), thereby circumventing the need for squash preparations for the purpose of chromosome analysis.

The exactness and simplicity of this peripheral method are best exemplified by its successful utilisation with a variety of vertebrate species. The diploid chromosome number in man has been confirmed as 46 with the use of peripheral leucocytes in short term cultures (Hungerford et al, 1959; Moorhead et al, 1960 and others). The somatic complements of several primates (Hamerton et al, 1961; Chu and Bender, 1962), various Felidae species (Hsu et al, 1963) and two equine species (Trujillo et al, 1962) have been correctly determined with the use of a similar technique. For the determination of Anuran karyotypes, the technique has been slightly modified (Beckert and Doyle, 1967).

Extremely low diploid chromosome counts were reported for some bovine species by earlier authors (von Bardeleben, 1892; Wodsedalek, 1920 and others). These low counts may have arisen from observations with inadequately preserved materials and possibly from the use of unreliable techniques. The recent use of in vitro peripheral blood cultures with several bovine species has revealed a diploid count of 60 (Sasaki and Makino, 1962; Basrur and Gilman, 1964; Gustavsson, 1966 and others).

Other tissue culture methods such as the short-term cultures from small biopsies (Edwards, 1960 and others) and the in vitro culture of

bone marrow cells (Nadler, 1964) have been successfully utilised in karyotype determination.

MATERIALS AND METHODS

The karyotypes of the two forms Bos taurus (L) and Bos (Bison) bison (L)were obtained by a peripheral-blood method, utilising phyto-hemagglutinin (PHA) stimulated lymphocytes.

A. Collection of Blood Samples

(1) Domestic Cattle -- Bos taurus (L)

15 ml. glass tubes (screw caps) containing 0.5 ml. of an anti-coagulant (heparin - 0.4 mg/ 10 ml. blood) and 0.25 ml. of an anti-biotic mixture (penicillin-streptomycin) were autoclaved for 20 minutes. During the slaughter of bulls and heifers at a local abattoir, blood samples from poleaxed beasts were collected in these sterile tubes.

(2) Bison Samples -- Bos (Bison) bison (L)

Glass tubes with rubber stoppers, containing 0.5 ml. anti-coagulant and 0.25 ml. antibiotic mixture were autoclaved similarly. Blood samples were collected in these sterile tubes, from the jugular veins of two young bison calves (male and female), from the herd at Elk Island National Park.

Both sets of samples were transferred to autoclaved 15 ml. grad-uated conical centrifuge tubes and were centrifuged at 800 r.p.m. for five to seven minutes (at room temperature), in an International Model HN centrifuge. These samples were then stored in the refrigerator at 4°C. for 40 to 48 hours. This procedure was found to yield a high mitotic index.

B. Culturing of Blood Samples and Culture Incubation

The culturing procedure was carried out under ethylene glycol conditions as a precaution against contamination. All equipment used in the procedure was autoclaved for 20 minutes.

Approximately 1.5 ml. plasma along with the buffy coat (layer of lymphocytes) was withdrawn with a sterile pipette, from the stored samples and diluted, in sterile, plastic T-flasks (Falcon), with a solution made up as follows: To 5 ml. TC medium 199 were added 2 ml. bovine serum (heat inactivated at 58°C. for 30 minutes), 0.25 ml. Penicillin-Streptomycin (100 units and 100 µgm respectively) and 0.25 ml. Bacto-Phytohemagglutinin (M or P) -- Difco Laboratories, Detroit.

The contents of the flask were swirled at room temperature to ensure thorough mixing. The cultures were then placed in a horizontal position within a Millipore Incubator and incubated at 38°C. (± 0.5°C.). Swirling of the cultures was continued periodically for about two hours. Temperature constancy was found to be an absolute pre-requisite for achieving desired results and therefore a constant temperature was maintained throughout the incubation period.

Following 70½ hours of incubation, colchicine (1 ml. of 0.008 g/ 100 ml. water) was added to the cultures. Sterile precautions beyond this stage of the procedure were found unnecessary. After the addition of colchicine, cultures were incubated a further four to five hours at the same temperature. The procedure of hypotonic pretreatment was begun immediately following the period of incubation in colchicine.

The contents of each flask were transferred to 15 ml. graduated conical centrifuge tubes and centrifuged at 800 r.p.m. for 15 minutes (room temperature or slightly higher than room temperature).

C. Hypotonic Pretreatment

A fresh solution of 0.8 per cent of sodium citrate was prepared and used as the hypotonic solution.

The supernatant was decanted from each centrifuge tube with a pipette. The "button" of cells at the bottom of each tube was resuspended in four to five ml. of the hypotonic solution. Resuspension was achieved by tapping the outside wall of the tube vigorously and by a gradual addition of hypotonic solution along the side of the tube. The cells were maintained in the hypotonic solution for 30 minutes and then centrifuged at 800 r.p.m. for 15 minutes, before the procedure of fixation was carried out.

D. Fixation

Freshly prepared fixative, consisting of three parts absolute methanol (4°C.) and one part glacial acetic acid was used. Optimal fixation was achieved when the amount of hypotonic surrounding the cells was minimal. Therefore most of the supernatant was decanted, without disturbing the "button" of cells at the bottom of the tube, before fixative was added. To avoid the clumping of cells, approximately 4 ml. fixative was added with a continuous agitation of the cell "button". This procedure of centrifugation, decantation and addition of fixative was repeated two more times. Resuspension of the cells after each cen-

trifugation and decantation was achieved by tapping the outside wall of the tube rather than by aspirating with a pipette. The cells were maintained in fixative for a total period of not less than 90 minutes. It was found that the total time in fixative could be varied up to 24 hours or longer, without deterioration in the preparation.

E. Slide Preparations

Precleaned glass slides were immersed in 70 per cent methanol overnight. Immediately following the removal of the last fixative, a suspension of cells was made in a small amount ($\frac{1}{2}$ ml.) of fresh fixative. A drop or two of this "hazy" suspension was then placed on a methanol-immersed, precleaned glass slide. The cells were allowed to spread on the slide and the methanol was ignited and allowed to burn off.

F. Staining

Various staining procedures *viz.* aceto-orcein, haematoxylin-eosin and Feulgen were tried. Preparations were stained with Feulgen since such preparations revealed a better morphology of the chromosomes. The procedure was as follows: Ignited slides were immersed in distilled H_2O for five minutes. The slides were drained on bibulous paper and then placed in 5N HCL (room temperature) for 40 minutes in a covered container. The slides were quickly rinsed in distilled water, prior to being placed in Feulgen stain in a dark, stoppered container for 30 minutes. Upon removal from the stain, they were rinsed in tap water until the water remained clear of purple stain. The slides were finally mounted in Gurr's water-mounting medium. Permanent prepara-

tions were dehydrated in alcohol, taken through xylol and then mounted in a permanent, synthetic mounting medium. On the average, four slides were made from each culture.

G. Microscopy

The slides were examined under a 10 x objective (Leitz Wetzlar microscope) for suitably spread metaphases. Chromosome counts of these spreads were done under a 45 x objective (Leitz Wetzlar), using a light green interference filter (546 m μ). The counting data are as tabulated in Table I.

H. Photography

Microphotographs were taken with a Leitz Ortholox microscope, using both a bright field, oil immersion system and a bright field, high dry system. The light source was an A.O. mercury arc lamp. A light green interference filter was used to obtain maximum contrast. The microphotographs were taken with a Leitz $3\frac{1}{4}$" x $4\frac{1}{4}$" bellows camera, at an extension length of 48 cms. The film used was Agepe FF, developed in a 1:2 Dektol: Water solution for four minutes. Contact prints were developed on a suitable grade of Kodak paper.

I. Karyotype Preparations

The karyotype of each animal was prepared by cutting chromosomes from enlarged photomicrographs (x 2000) and arranging them in pairs on the basis of their overall size.

J. Estimation of Relative Percentage Lengths

For the purpose of karyotypic comparison, the percentage length

of each chromosome relative to the total length of a haploid set was computed. First the lengths of the individual chromosomes in each of the four karyotypes, were approximated in millimetres with a pair of dividers. These individual chromosome lengths were then added together to obtain a diploid genome length for each karyotype. The haploid genome length was calculated by halving the value for the diploid genome length (See Table II). Finally, the relative length for each pair of chromosomes, in each of the four karyotypes was computed by dividing the average length of each pair of chromosomes by the haploid genome length. Our estimationsof the relative percentage lengths of the various chromosomes were based on measurements of a single cell in each of the four cases; the relative percentage lengths are tabulated in Table III.

RESULTS

TABLE I

DISTRIBUTION OF CHROMOSOME NUMBERS IN CELL COUNTS OF CATTLE AND BISON

Animal ↓ / # of cells →	Chromosome Number						Total # of cells counted	Modal Chromosome #
	60	59	58	57	56	55		
♂ cattle # of cells	26	4	1	-	1	-	32	60
♀ cattle # of cells	29	4	2	1	3	1	40	60
♂ bison # of cells	25	3	1	2	-	-	31	60
♀ bison # of cells	34	6	4	2	2	2	50	60

The modal number of chromosomes per cell, of both males and females of the two species is 60, consisting of 58 autosomes and 2 sex chromosomes. This is in agreement with the findings of other investigators (Melander, 1959; Sasaki and Makino, 1962; Basrur and Moon, 1967 and others). Hypermodal counts for both males and females of the two forms were observed but these were few in number and were therefore not included in Table I.

Karyotype Analysis

The majority of the chromosomes in the two forms, although differing in length, are morphologically similar viz., rod shaped structures with near-terminal centromeres. The sex chromosomes differ from the autosomes morphologically and may therefore be placed in a separate group in the karyotypes (See Figures 1, 2, 3 and 4). A single exception is the Y-chromosome of the bison, which resembles in morphology one of the smallest autosomes.

The autosomes have been arbitrarily divided into five groups, on the basis of overall size. They have been assigned the following descriptive terms:-

Row 1 - Long telocentrics

Row 2 - Medium long telocentrics

Row 3 - Medium telocentrics

Row 4 - Medium short telocentrics

Row 5 - Short telocentrics.

In reality the autosomes may have a very short arm and therefore be true acrocentrics. However, a short arm as a morphological characteristic was not detected in all the autosomes of our preparations. Therefore, they were assigned the term "telocentrics".

The precise lengths of the individual chromosomes were not measured, since the four karyotypes do not appear to be uniform in chromosome contraction. This is in all likelihood a result of the different cells (from which the karyotypes were prepared), being exposed to colchi-

cine treatment for varying periods of time. Instead the haploid genome length for each karyotype was estimated and on the basis of the respective haploid genome values, the relative percentage length for each pair of chromosomes in the four karyotypes was computed (See Table III). Our estimations of the relative percentage lengths of the various chromosomes, although based on measurements of a single cell in each case, are reasonably comparable to similar estimations by Basrur and Moon (1967).

A visual comparison of the karyotypes of the two species reveals the following:

(1) The short telocentrics are approximately one-third the size of the long telocentrics.

(2) The X-chromosomes are of the sub-metacentric type and are approximately of the same size as the long telocentrics.

(3) The Y-chromosomes are similar in size to the short telocentrics. However, in the domestic cattle, the Y-chromosome differs morphologically from the short telocentrics. It is a true short metacentric while the Y-chromosome of the bison resembles the short telocentrics both in morphology and size.

TABLE II

ESTIMATION OF CHROMOSOME LENGTHS
AND HAPLOID GENOME LENGTHS

Male Bison

Row Number	Individual Chromosome Lengths (mm.)											
Row 1	8.5	8	8.5	7.5	8	7	7	7	6.5	6.5		
Row 2	6.5	6.5	6	6.5	6	6	6	6	5.5	5.5	5.5	5.5
Row 3	5	5	5	5	4.5	4.5	4.5	4.5	4.5	4	4.5	4
Row 4	4.5	4	4	4	4	3.5	4	3	3.5	3.5	3.5	3.5
Row 5	3	3.5	3.5	3	3	3	3	3	3	2.5	2.5	2.5
Sex Chromosomes							7	3				

Diploid Genome Length = 291.5

Haploid " " = 145.8

Female Bison

Row Number	Individual Chromosome Lengths (mm.)											
Row 1	10	10	10	9	9	9	8.5	8	8	8		
Row 2	8	8	8	7.5	7.5	7.5	7.5	7	7	7	6.5	6.5
Row 3	6.5	6.5	6.5	6.5	6	6	5.5	5.5	5.5	5.5	5.5	5.5
Row 4	5	5	5	5	5	4.5	4.5	4.5	4	4	4	4
Row 5	4	4	4	4	3.5	3.5	4	3	3.5	3.5	3	3
Sex Chromosomes							9.5	9.5				

Diploid Genome Length = 365.0

Haploid " " = 182.5

TABLE II (Continued)

ESTIMATION OF CHROMOSOME LENGTHS
AND HAPLOID GENOME LENGTHS

Male Cattle

Row Number	Individual Chromosome Lengths (mm.)											
Row 1	11	11	9.5	9.5	10	9	10	9	9	9		
Row 2	9	9	8	8	8	8	8	8	8	8	7.5	7.5
Row 3	7	7	6.5	6.5	6	6	6	6	6	6	6	5.5
Row 4	5.5	5.5	5.5	5.5	5.5	5.5	5	5	5	5	5	5
Row 5	5	4.5	4.5	4.5	4.5	4.5	4	4	4	3.5	3.5	3
Sex Chromosomes							11	4				

Diploid Genome Length = 396.0

Haploid " " = 198.0

Female Cattle

Row Number	Individual Chromosome Lengths (mm.)											
Row 1	9	8.5	8	7.5	7.5	7.5	7	7	7	7		
Row 2	7	6.5	6.5	6.5	6.5	6	6	6	6	6	5.5	5.5
Row 3	5	5	5	5	5	5	5	4.5	4.5	4.5	4.5	4.5
Row 4	4.5	4.5	4	4	4	4	3.5	3.5	3.5	3.5	3.5	3.5
Row 5	3.5	3.5	3.5	3.5	3.5	3	3	3	3	3	3	2.5
Sex Chromosomes								9	8.5			

Diploid Genome Length = 309.0

Haploid " " = 154.5

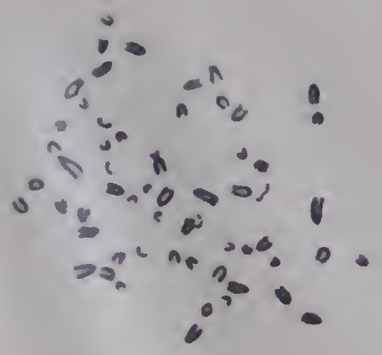
TABLE III

RELATIVE PERCENTAGE LENGTHS FOR
EACH PAIR OF CHROMOSOMES

Chromosome Pair Number		Bison		Cattle	
		Male	Female	Male	Female
Row 1	1	5.6	5.7	5.7	5.5
	2	4.8	5.0	5.5	5.2
	3	4.8	4.8	5.1	4.9
	4	4.8	4.5	4.8	4.5
	5	4.5	4.5	4.5	4.4
Row 2	6	4.5	4.4	4.5	4.4
	7	4.0	4.2	4.3	4.2
	8	4.0	4.0	4.1	4.1
	9	4.0	3.9	4.1	4.0
	10	4.0	3.9	3.8	3.8
	11	3.8	3.6	3.8	3.6
Row 3	12	3.5	3.2	3.4	3.6
	13	3.3	3.2	3.4	3.6
	14	3.0	3.2	3.1	3.3
	15	3.0	3.1	3.1	3.0
	16	3.0	2.9	2.9	3.0
	17	2.9	2.9	2.9	3.0
Row 4	18	2.8	2.9	2.9	2.7
	19	2.8	2.6	2.8	2.7
	20	2.8	2.6	2.6	2.6
	21	2.5	2.3	2.4	2.5
	22	2.5	2.3	2.4	2.2
	23	2.5	2.3	2.4	2.2
Row 5	24	2.4	2.3	2.2	2.2
	25	2.3	2.3	2.2	2.2
	26	2.3	2.1	2.1	1.9
	27	2.0	1.9	2.1	1.9
	28	1.9	1.9	1.9	1.9
	29	1.6	1.8	1.7	1.6
Sex Chromosomes	30	5.6 1.9	5.8 5.4	4.8 2.1	5.2 5.2

Figure 1: The karyotype of a male bison as prepared from an enlarged photomicrograph of the cell shown in the top right hand corner.

800 X



Row 1

Row 2

Row 3

Row 4

Row 5

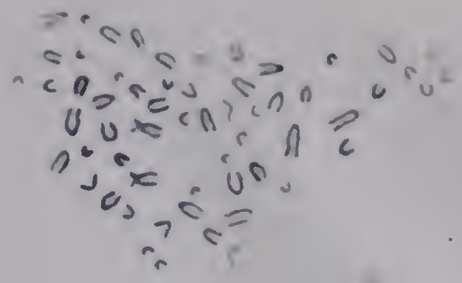
Sex chromosomes

2000 X

10 μ

Figure 2: The karyotype of a female bison as prepared from an enlarged photomicrograph of the cell shown in the top right hand corner.

800 X



Row 1

Row 2

Row 3

Row 4

Row 5

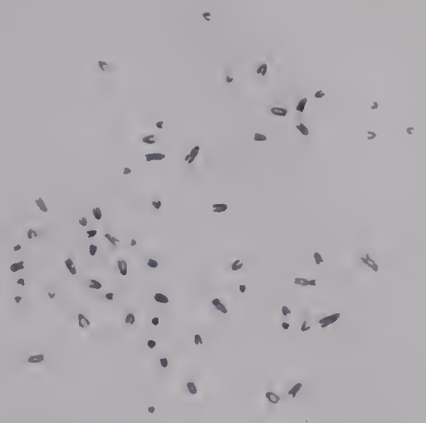
Sex chromosomes

2000 X

10 μ

Figure 3: The karyotype of a domestic male as prepared from an enlarged photomicrograph of the cell shown in the top right hand corner.

500 X



Row 1

Row 2

Row 3

Row 4

Row 5

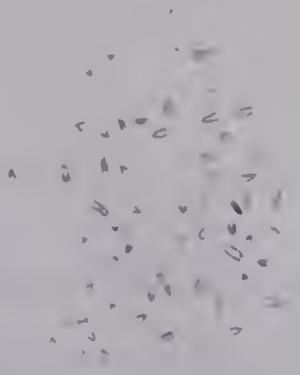
Sex chromosomes

2000 X

10 μ

Figure 4: The karyotype of a domestic female as prepared from an enlarged photomicrograph of the cell shown in the top right hand corner.

800 X



Row 1

Row 2

Row 3

Row 4

Row 5

Sex chromosomes

2000 X

10 μ

DISCUSSION

The karyotypes of both the domestic cattle and the American bison, are asymmetrical (the term as defined by Stebbins, 1950, implies that there are several telocentric chromosomes in the complement and there is a wide variation in their size). Although the majority of chromosomes are morphologically similar, some variation in the location of the centromeres was observed. However, the differences were too small to be used for the purpose of identifying specific autosomes. The presence of satellites was not detected on any of the chromosomes. Chromatid gaps were observed in a few chromosomes but these were inconsistent and therefore could not be used as a basis for identification. Thus, none of the autosomes could be individually identified with any certainty. The karyotype arrangements are arbitrary and the pairing of chromosomes within each group implies that we may consider the chromosomes to be homologous. However, since the chromosomes within each group are strikingly similar and the identifying characteristics few, the chromosomes paired within each group may, in fact, not be homologous. Similarly, chromosomes placed in identical positions in the karyotype arrangements of the two bovines, do not necessarily imply that we consider them to be "absolutely" homologous (or homeologous).

Taxonomic Status of the American Bison and the Domestic Cattle

A "species" is defined as a group of populations (usually constituting a sub-division of a taxonomic group known as genus), having certain common and permanent characteristics which clearly distinguish

it from other groups; and the gene exchange between such groups is limited or prevented in nature by one or more reproductive isolating mechanisms (Dobzhansky, 1937).

In considering problems of karyotype evolution, special significance is attached to instances where species of different genera or sub-genera, are found to possess similar karyotypes. Such findings prompt several questions: First, are these forms actually members of different genera or are they related closely enough to be grouped within one genus? Second, if they are related closely enough should they be classified as different species of a single genus? For a definite establishment of chromosome homology between groups, it is essential that, the meiotic chromosome behaviour in hybrids between the groups, be studied. Such studies may provide precise answers to some of the above questions and may lead to a revision of the taxonomic status of the groups under consideration.

On the basis of morphological criteria alone, the domestic cattle and the American bison have been classified into distinct sub-genera, Bos and Bison respectively (Audubon and Bachman, 1851; Smith, 1827). In fact, such a classification, on the basis of morphological criteria, is true of several bovine ruminants - oxen, bisons, yaks and buffaloes (Audubon and Bachman, 1851; Smith, 1827 and others). Our karyotype comparison indicates that the domestic cattle and the American bison possess chromosome complements that are strikingly similar. The identity in number and similarity in morphology, suggest that the two

ungulates are closely related. There is some question then, whether the morphological differences between the two bovine forms justify the establishment of distinct sub-genera.

Recent observations reveal that the various bovine ruminant forms can interbreed to produce viable hybrid offspring. Nevertheless, the fertility of these hybrid offspring in most cases is very much reduced (Zeuner, 1963). For example, the bison and the cattle have been known to interbreed and give rise to hybrid progeny, often referred to as "cattalos" (Boyd, 1914; Peters, 1964). On the basis of this ability to interbreed and produce viable hybrid offspring, the various bovine ruminants have been grouped into a single genus Bos. The striking similarity in the two karyotypes as revealed by the karyotypic comparison, justifies the grouping of the bison and domestic cattle within the genus Bos. On the basis of Dobzhansky's definition of a species, the two bovines are indeed separate species. However, confirmatory meiotic studies in the hybrid between the two groups are necessary to validate the above conclusion. If indeed such studies indicate that they are separate species, then their similarity in karyotypes must have originated from an immediate common ancestor.

Mechanisms of Karyotype Evolution

In the animals it is generally accepted that almost all evolutionary changes have had their origin in the chromosomes. A gross alteration in the structure or behaviour of these entities that constitute the physical basis of heredity, represents an evolutionary

change.

On the basis of our karyotypic comparison alone, it was impossible to detect the different mechanisms that can be associated with evolutionary change. Gross structural rearrangements such as centric fusions (or Robertsonian translocations) and centric dissociations, two mechanisms of karyotype evolution, fairly common in mammals (Chu and Swomley 1961; Nadler, 1964; Matthey, 1964 and others), were not detected in this study.

Polyploidy and differential polyteny, two other mechanisms associated with evolutionary change (Hughes-Schrader, 1953), were not detected either. The former represents an evolutionary change in the number of chromosomes while the latter signifies a change in the number of constituent, longitudinal strands, of which the individual chromosomes are composed. It must be emphasized that these mechanisms of karyotype evolution have been in operation and are best detected cytophotometrically (Hughes-Schrader, 1953). The phenomenon of polyploidy explains reasonably well the evolution of most plant karyotypes, while in animals it has been infrequent (White, 1954). Polyteny, on the other hand, readily explains the evolution of certain insect karyotypes (Schrader and Hughes-Schrader, 1956; Hughes-Schrader, 1958).

Feulgen cytophotometry involves the measurement of the relative amount of DNA per nucleus (Caspersson, 1936; Pollister and Ris, 1947). This amount of DNA is relatively constant for most of the nuclei of a species. Cytophotometric measurements of this DNA value in equivalent

nuclei of different species should provide an index of the total DNA content of the different karyotypes (Hughes-Schrader, 1953). Cytophotometry detects both polyploidy and polyteny as multiple DNA values, i.e. when DNA values of closely related species, uniform in chromosome number, represent multiples of a basic amount, then the above mechanisms of karyotype evolution are indicated to be in operation. In situations where structural rearrangements mask evolutionary homologies, Feulgen cytophotometry has provided a more convincing explanation of interspecific relationships (Hughes-Schrader, 1951, 1953). The use of cytophotometry as a criterion in assessing the taxonomic relatedness of different species appears justified; and it is best exemplified by its extensive use in the establishment of phylogenetic relationships in several insect forms (Hughes-Schrader, 1958; Wahrman and O'Brien, 1956 and others).

Several structural changes have failed to become established in the course of evolution because they have been genetically deleterious. Satisfactory rearrangements are such that they do not cause a genic imbalance. They do not diminish the mechanical efficiency of mitosis and meiosis and can therefore become established (White, 1954). The two karyotypes -- Bos taurus and Bos bison differ with respect to the Y-chromosomes of the heterogametic sexes. Although structural rearrangements such as deletions were not detected in our study, indications are that the bison Y-chromosome has evolved as a result of a heterochromatic deletion in the Y-chromosome of domestic cattle. The

deletions that have become established in phylogeny are heterochromatic in nature (White, 1954). If such a deletion is the only cytological (or detectable karyotypic) change, then it is suggested that the gross morphological differences between the two forms (viz. pelage differences, structural and anatomical differences, etc.), are due to genic differences in the two karyotypes; the genes for the various morphological characters being located in all probability on chromosomes other than the sex chromosomes. A second possibility is that the two karyotypes have evolved separately from a common ancestor.

The Y-Chromosome of the Bison

The Y-chromosome of the bison is a telocentric, indistinguishable from the smallest telocentric autosomes. Unequivocal identification of the bison Y-chromosome, it is hoped, may be achieved through labelling studies. Studies of replication patterns of bovine sex-chromosomes in cell cultures indicate that the Y-chromosome is late replicating (Gartler and Burt, 1964, 1965). It begins to replicate its DNA two or more hours later than the other chromosomes in the complement. If tritiated thymidine is introduced into a growing culture near the end of the DNA-synthesis period, the Y-chromosome should be more heavily labelled at mitosis than the other chromosomes and therefore be positively identified.

Conclusion

Our karyotype comparison suggests that the two members of the family Bovidae, Bos taurus and Bos bison, are closely related species

(if not members of the same species). For a definite establishment of their chromosomal homologies and species classification, it is essential that the meiotic behaviour of the chromosomes of the hybrid between the two groups ("cattalo") be studied.

Karyotypic comparisons enable a direct comparison of the dimensions and morphology of the mitotic complements of the species being studied. They permit the detection of gross structural alterations in the chromosomes. Investigations of a cytotaxonomic nature, when based on a variety of criteria, can approach a "true realism and naturalism" (Matthey, 1951). Therefore it is suggested that, if the taxonomic relatedness of two forms is to be demonstrated effectively, then a comparative chromosome morphological study be complemented with a cytophotometric study. Data from these two types of analyses, when jointly considered should enable a better assessment of the cytotaxonomic relationship(s) between Bos taurus and Bos bison. Apart from karyotypic comparisons and cytophotometric studies, it is now possible to demonstrate phylogenetic relatedness by measuring the degree of hybridisation between the DNA molecules of different species (McCarthy and Bolton, 1963; Dutta et al, 1967). Such a criterion has been successfully applied to the plant kingdom and its validity as a taxonomic criterion is being tested with other organisms (Dutta et al, 1967).

Thus, comparative cytological studies may enable cytotaxonomists to confirm or negate the conclusions of the experienced systematists. However, it must be borne in mind that cytology alone will not unravel the solutions to all taxonomic problems, as some have supposed.

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